

# A Genetic Based Approach to Management and Stocking of Muskellunge

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Fish stocking has long been used as a management tool among fisheries biologists and continues to be met with popular public opinion. Despite perceived simplicity, the full effects of stocking remain cryptic for many fish populations. Stocking has frequently been used to maintain or establish successful muskellunge (*Esox masquinongy*) populations. Coupled with other management practices the Minnesota Department of Natural Resources (MNDNR) has established Minnesota as a premier muskellunge fishery. From 1958 to 2012 the MNDNR stocked three strains of muskellunge in Big Mantrap Lake, MN. The first from Shoepack Lake, MN (hereafter Shoepack-strain) was used until the realization that fish were not attaining sizes sought after by anglers. The MNDNR then made a statewide switch to a source from Wisconsin before developing a source from Leech Lake, MN (hereafter Leech-strain). Using 13 microsatellite markers, genetic contributions of each source population were estimated from samples taken between 1984 and 2013 in Big Mantrap Lake. Analysis of current and past genetic makeup of Big Mantrap Lake demonstrated a successful dilution of the Shoepack-strain from 96% in 1984 to 11% in 2013 as the Leech-strain made up 85% of the genetic makeup in the most recent sample. This study reinforces the use of genetic information as a management tool in evaluating ancestry of stocked fish. These findings will help the MNDNR make management decisions related to size structure and genetic makeup of muskellunge populations.

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## Introduction

Stocking has long been used as a management technique among fisheries biologists (Halvorson 2008). This is partially due to the popular public perception of its simplicity; stocking will result in an increase or maintenance of the current catch rates (Travis et al. 1998, Welcomme and Bartley 1998). Currently the exact contribution of stocked fish remains uncertain for many species and its effects are not fully understood (Schramm and Piper 1995; Nickum et al. 2005).

Stocking has been used to restore and/or enhance native populations of muskellunge (*Esox masquinongy*) and remains a popular management tool even in regions with abundant populations (Margenau 1999; Kerr 2007; Wingate and Younk 2007). Coupled with other management tools (size/seasonal harvest restrictions, catch and release, etc.) stocking has been used as a way to establish successful fisheries. Minnesota remains a premier muskellunge fishery due to the use of these tools to

maintain and expand the population of these trophy fish (Wingate and Younk 2007).

Big Mantrap Lake is one of the lakes currently stocked and managed by the Minnesota Department of Natural Resources (MNDNR) for muskellunge. Located in the Park Rapids area within the state of Minnesota, it is a 654 ha lake with a maximum depth of 20 m. Big Mantrap, like other lakes in the area can be described as a class 25 lake (based on the Minnesota Department of Natural Resources lake classification program). Lakes in class 25 are deep and clear with an irregular shape (MNDNR unpublished data). In the past stocking has included fry, fingerlings, and yearlings, however, more recently approximately 750 fingerlings have been stocked in the lake bi-annually in even years (Appendix A).

Muskellunge stocking in Big Mantrap Lake began with a strain derived from Shoepack Lake (hereafter, Shoepack-strain). The Shoepack-strain was stocked in the lake from 1958 to 1983 (Appendix

A). After experimentation that showed a genetic predisposition for slower growth rates and smaller maximum size for the Shoepack-strain muskellunge, stocking of the strain throughout Minnesota was discontinued (Younk and Strand 1992). After this study the MNDNR switched to a privately raised strain of muskellunge from an unknown Wisconsin source (hereafter, Wisconsin-strain). Big Mantrap was only stocked with Wisconsin strain in 1987; from 1988 to present, a strain of muskellunge derived from Leech Lake (hereafter, Leech-strain) has been used to stock the lake (Appendix A). This unique stocking history allows a chance to observe the genetic contributions of each strain to the current and past muskellunge population in the lake. Contributions such as these are of great interest to both fisheries managers and anglers due to the decreased desirability (based on growth rate and maximum size) of Shoepack-strain and the attempt to dilute out the effects of its stocking with the more desirable Leech-strain.

*Objectives-* The observable genetic makeup of a population of muskellunge can be attained and categorized (Kapusinski et al. 2013). Previous research has shown that the use of samples from a source population of stocked fish can be used to determine the ancestry of muskellunge in a population (Miller et al. 2009, Miller et al. 2012). This study will determine (1) the current ancestry of the population in Big Mantrap based on sampling conducted in 2013, (2) estimate the change in the genetic makeup of muskellunge in the lake as source populations have changed, and (3) determine whether stocking of more desirable strains has substantially diluted out Shoepack-strain fish.

## Methods

*Sample Collection-* All scales processed during the study were obtained by local MNDNR biologists during the 2013 spring assessment of muskellunge spawning on Big Mantrap Lake using modified Fyke nets with 152 x 183 cm-frame and 30.5 m leads. Procedures for the capture and processing of samples prior to 2013 can be found in Miller et al. (2009, 2012). These include all source samples from Leech Lake (Leech-strain source), Shoepack Lake (Shoepack-strain source), and Tomahawk Lake (Wisconsin-strain source). All sample sizes in the study were based on scale availability and budget restrictions. Though sample sizes vary (1984 n = 39, 2004 n = 47, 2013 n = 133) comparisons can be made between samples based on percent of ancestry in populations.

*Genotyping-* All 133 scales processed during the study were prepared in the following way. A 5% (weight/volume) solution of chelating resin (Chelex,

Sigma Chemical, St. Louis, Missouri) was prepared. Scales were then cut (due to size) and half was placed in a 1.5 mL tube with 250  $\mu$ L of the solution. Samples were placed in a 56  $^{\circ}$ C water bath overnight and boiled for eight minutes before preparation for polymerase chain reaction (PCR). The 14 loci described Sloss et al. (2008) were used with the following changes. The microsatellite locus EmaA5 was not used in order for the remaining loci to combine together in a single electrophoresis run as described in Miller et al. (2012). The primer EmaD126 was used in place of EmaD4 as described in Miller et al. (2009). PCR preparation was done in accordance with Miller et al. (2009) with microsatellite amplification performed in 15  $\mu$ L reactions containing 1  $\times$  polymerase buffer (10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100), 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each deoxynucleotide triphosphate, 0.5  $\mu$ M of the forward and reverse primers, and 0.5 units of Taq DNA polymerase (Promega, Madison, Wisconsin). A water blank was included in each set of samples to detect possible contamination of PCR solutions. A thermocycler (Hybaid Omn-E; Thermo-Hybaid U.S., Franklin, Massachusetts) was then used to carry out the amplification process. For each PCR plate 35 cycles were run at the following temperature profile: 95  $^{\circ}$ C for 30 s, 50  $^{\circ}$ C for 30 s, and 72  $^{\circ}$ C for 1 min, followed by a 20 min extension at 72  $^{\circ}$ C. PCR plates were then screened via gel electrophoresis for success by randomly picking wells to test before pooling. Plates were then pooled into a single plate for analysis in the following amounts: 2  $\mu$ L of EmaA10/EmaD12a, 2  $\mu$ L of EmaC1/EmaD126, 4  $\mu$ L of EmaA11/EmaB110, 3  $\mu$ L of EmaD5/D116, 4  $\mu$ L of EmaD6, 2  $\mu$ L of EmaA102, 2  $\mu$ L of EmaA104, 2  $\mu$ L of EmaD114, and 2  $\mu$ L of EmaB120. The pooled plate was then submitted to the University of Minnesota Genomics Center (St. Paul, MN) for electrophoresis on an ABI Prism 3130x1 genetic analyzer (Applied Biosystems, Foster City, California). The core facility then returned data files and the program GENEMAPPER V4.1 (Applied Biosystems) was used to score alleles.

*Population Ancestry-* In order to determine the number of genetically distinct populations in our samples, we used a Bayesian clustering algorithm program STRUCTURE (version 2.3.4; Pritchard et al. 2000; also refer to: pritch.bsd.uchicago.edu). To determine the number of distinct populations (K) in our data set we ran five independent replicates for each K. We ran the replicates from K=1 (which would indicate a single population) to K=10 (greater than the known number of populations of stocked fish plus a native population in Big Mantrap Lake). The burn-in period for each replicate was 50,000

replications, followed by 250,000 Markov-chain Monte Carlo simulations run underneath a model that assumed admixture and correlated allele frequencies. STRUCTURE HARVESTER (version 0.6.94; Dent and vonHoldt 2012; also refer to: [taylor0.biology.ucla.edu/struct\\_harvest/](http://taylor0.biology.ucla.edu/struct_harvest/)) was then used to estimate K using the Evanno method. The mean and standard deviation of likelihood estimates were used ( $\Pr[X|K]$ =the posterior probability of the data given K populations) among the runs for each value of K. Coupled with the knowledge of the stocking history on Big Mantrap, we were able to determine the most likely value of K.

*Individual Ancestry*- One of the replicates from the most likely K was used to evaluate the ancestry of individual fish within the samples. For each given K STRUCTURE runs algorithms to estimate the proportion of ancestry (q) in the genome. We used these estimates of ancestry to compare individuals and then to compare ancestry among populations by averaging q among individuals within each sample year. STRUCTURE typically produces nonzero estimates for ancestry from all populations, even when ancestry is unlikely (e.g., Wisconsin-strain ancestry in the Leech-strain source population). We assigned ancestry based on a 90% rule (A single source population is indicated by  $\geq 90\%$  ancestry). This was determined by calculating the greatest percentage at which the known source population samples would assign to their respective population with  $<10\%$  error.

*Ancestry and Fish Size*- The relationship between individual ancestry and fish size was evaluated for our most recent sample. We emphasized the impact of Shoepack-strain ancestry on the length of each fish sampled (due to the reduced desirability of Shoepack-strain fish). We were unable to compare growth rates for the fish within our sample years due to the difficulty associated with aging muskellunge (especially older fish) via scales (Fitzgerald et al. 1997). Instead the frequency of male and female muskellunge with and without Shoepack-strain ancestry in eight length classes was calculated. Comparisons between distributions of the four groups (males with Shoepack-strain ancestry, males without Shoepack-strain ancestry, females with Shoepack-strain ancestry, and females without Shoepack-strain ancestry) were made. To do this a Shapiro and Wilk's test for normality was conducted on all four groups and p-values  $< 0.1$  were considered non-normal distributions (Royston 1995). The findings of these tests were then confirmed using Q-Q plots in program R. Due to the fact that non-normal distributions would be included in each comparison a Mann-Whitney U test was then run for each sex to

test for differences between individuals with and without Shoepack-strain ancestry (Hollander and Wolfe 1973). A comparison between the proportion of individuals in each group above and below the 1219 mm total length (TL) limit for harvesting muskellunge on Big Mantrap Lake was also made for samples taken in 2004 and 2013. Mean total lengths (mm) for male and female muskellunge samples from 1993 to 2013 were calculated and compared using regression analysis in order to determine whether mean total length had increased as stocking of Leech strain fish increased.

## Results

*Number of Observed Populations*- Output from STRUCTURE along with knowledge of prior stocking history on Big Mantrap Lake lead to the conclusion that three genetically distinct populations contributed to the ancestry of the fish in the sample. When run through STRUCTURE HARVESTER for five repeated simulations with values of K ranging from 1-10, the highest average likelihood estimated as  $\log_e \Pr[X|K]$  was observed at K=7. However upon examination of the data a dramatic increase in likelihood was observed as K increased from 1 to 3 (Figure 1). Pritchard et al. (2010) describes this leveling off as an observable pattern and suggests that true K is found at the inflection where likelihoods begin to plateau. Coupling this plateau with the low observed standard deviation at K=3 and the increase in standard deviation subsequently as values of K increased from 4 to 10, a K of 3 is the most likely fit to the stocking history of Big Mantrap Lake.

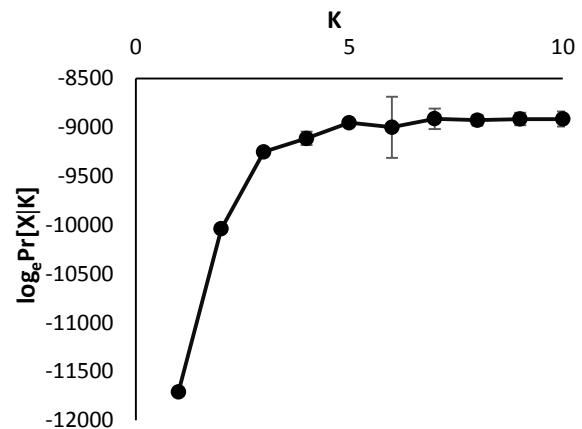


Figure 1- Mean ( $\pm$ SD) of the posterior probability of the data given K clusters ( $\log_e \Pr[X|K]$ ), across five replicate simulations with K-values of 1-10.

Though a native muskellunge population existed on Big Mantrap Lake, we were unable to find indications that they persisted in any measureable amount after the stocking events that have taken

place. In conclusion, though no clear observation of the native Big Mantrap muskellunge population can be made we can observe the three stocked populations: Leech-strain, Wisconsin-strain, and Shoepack-strain.

We compared the number of populations identified by STRUCTURE HARVESTER to ancestry proportion (q) output from STRUCTURE using our source samples. These are the known populations from Shoepack Lake, Leech Lake, and from Tomahawk Lake (in the region thought to include the source population used to found Wisconsin-strain). Hereafter q values will be subscripted with the source population (i.e., Shoepack Lake ancestry will be written  $q_S$ ). Individuals from the Shoepack Lake sample showed a strong affinity for one population with  $q_S \geq 0.90$  for all but one fish that had a  $q_S$  of 0.81. Two source samples were run from Leech Lake. The first also showed a strong affinity for one population with  $q_L \geq 0.90$  for all but one fish that had a  $q_L$  of 0.89. The second showed affinity with  $q_L \geq 0.90$  for all but six fish with  $q_L$  values ranging 0.57 to 0.86. Tomahawk Lake showed strong affinity with  $q_W \geq 0.90$  for all but three fish with  $q_W$  values from 0.82 to 0.86.

*Ancestry by Sample-* Overall genetic ancestry from each source strain was calculated for each of the sample years. The earliest available sample from 1984 collected 28 years after the initial stocking of Shoepack-strain contained 96% Shoepack-Strain and 4% of native Big Mantrap muskellunge ancestry (Figure 2). The 2004 sample was collected 17 years after the stocking of Wisconsin-strain and 18 years after the initial stocking of Leech-strain. This sample contained only 19% Shoepack-strain ancestry and the rest of the sample was composed of 59% Leech-strain and 22% Wisconsin-strain (Figure 2). The most recent sample from 2013 was dominated by Leech-strain ancestry (85%), with only 4% Shoepack-strain and 11% Wisconsin-strain measured in the sample (Figure 2).

*Individual Ancestry-* Individual ancestry proportions (q) were calculated for each sample and pure ancestry as well as admixture were estimated for all individuals. The muskellunge from the 1984 sample were a majority pure Shoepack-strain (87%) (Table 1). Though individuals designated as crosses with Leech or Wisconsin-strain ancestry were observed in this sample, it is unlikely that these could exist because that these strains were not introduced until after this sample was collected (Appendix A). Therefore, we attribute these observations to remnant genetic material from the native Big Mantrap muskellunge population. Fish in the 2004 sample had the most variability with 49% pure Leech-strain, 15% pure Wisconsin-strain, and 6% pure Shoepack-strain

observed (Table 1). All possible crosses between strains made up the remaining 30% of the sample (Table 1). The 2013 sample was a majority Leech-strain (71%) with only one other pure Wisconsin-strain fish. A change in admixture was observed in the most recent sample, most notably an increase in L x W crosses and a lack of S x W crosses (Table 1).

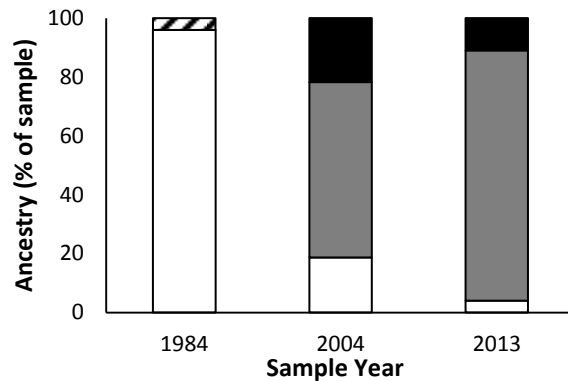


Figure 2- Mean ancestry of muskellunge from Big Mantrap Lake, MN, for samples from three assessments from 1984 to 2013. White proportions of bars correspond to Shoepack-strain ancestry, grey to Leech-strain, black to Wisconsin-strain, and the striped pattern to native Big Mantrap muskellunge.

Table 1- Individual ancestry of muskellunge from Big Mantrap Lake, MN, in samples from three assessments from 1984 to 2013. Shown are the number of individuals and the percent of each sample estimated to have pure ancestry from the Leech-strain (L), the Wisconsin-strain (W), and the Shoepack-strain (S). Also included are admixed individuals with ancestors from sources indicated in the headings.

Ancestral Source(s)	Sample Year		
	1984	2004	2013
L	0 (0%)	23 (49%)	94 (71%)
S	34 (87%)	3 (6%)	0 (0%)
W	0 (0%)	7 (15%)	1 (1%)
L x S	2 (5%)*	3 (6%)	9 (7%)
L x W	0 (0%)	4 (9%)	26 (20%)
S x W	2 (5%)*	5 (11%)	0 (0%)
L x S x W	1 (3%)*	2 (4%)	3 (2%)

\*Due to the fact that only Shoepack-strain was introduced at this time these admixed individuals are attributed to ancestry from the native Big Mantrap muskellunge population.

*Shoepack ancestry over time-* Due to the interest in Shoepack-strain fish we looked at all indicators of Shoepack ancestry over time. Overall Shoepack ancestry decreased over time with mean ancestry decreasing from 96% in 1984, to 19% in 2004, and finally down to 4% in 2013 (Figure 2). Number of pure and admixed Shoepack-strain individuals sampled each year also decreased. We found a majority pure Shoepack-strain ancestry (87%) in the 1984 sample with a small amount of the native Big Mantrap Lake muskellunge. Pure Shoepack-strain ancestry decreased to 6% in 2004 with 10 admixed individuals showing some Shoepack-strain ancestry (i.e., L x S, S x W, or L x S x W). No pure Shoepack-strain ancestry was observed in the 2013 sample but 12 Shoepack-strain crosses were observed (Table 1). These changes in the proportions of Shoepack-strain individuals can be observed in  $q_s$  values as well. Ancestry proportion ( $q$ ) for individuals decreased over time with  $q_s$  values ranging between 0.99 and 0.60 in 1984, to  $q_s$  values from 0.99 to 0.01 in 2004, and  $q_s$  values not getting above 0.55 in 2013 (Figure 3).

*Ancestry and Fish Size-* The relationship between ancestry and fish size was examined for our most recent sampling event by comparing lengths of individuals with Shoepack-strain ancestry to individuals with other ancestry (i.e., pure Leech-strain, L x W, etc.). Though no pure Shoepack-strain individuals were captured in the most recent sample a number of admixed individuals were (Table 1, Figure 3). All individuals were grouped into eight length classes ranging from <965 mm to  $\geq 1270$  mm TL and plotted against each other. The greatest number of females with Shoepack-strain ancestry (2) occurred in both the <965 mm and 1067-1117 mm TL classes; the class with the highest number other ancestry (8) occurred in the 1016-1066 mm TL class (Figure 4). The greatest number of males with Shoepack-strain ancestry (3) was found in the <965 mm TL class; the class with the greatest number of other ancestry (21) occurred in the 965-1015 mm TL class (Figure 4). Females with shoepack-strain ancestry was the only group from the 2013 sample to have a normal distribution. Other females ( $W = 0.93$ ,  $p = 0.01$ ), other males ( $W = 0.96$ ,  $p = 0.03$ ), and males with shoepack strain ancestry ( $W = 0.78$ ,  $p = 0.06$ ) all had non-normal distributions. In the 2013 sample the median length for Shoepack-strain females (1098 mm) was less than females without (1133 mm) though no statistical difference could be found ( $W = 126$ ,  $p = 0.27$ ). Likewise, the median length for Shoepack-strain males (958 mm) was less than males without (1009 mm) though no statistical difference could be found ( $W = 112.5$ ,  $p = 0.34$ ).

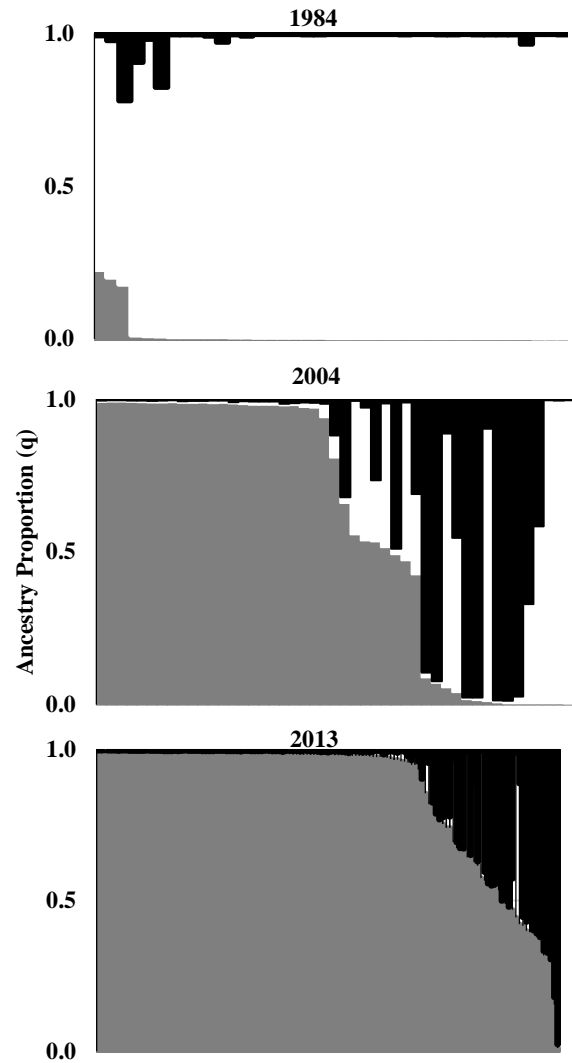


Figure 3- Individual ancestry proportion ( $q$ ) of muskellunge from Big Mantrap Lake, MN, in samples from three assessments from 1984 to 2013. Individuals are represented vertically and the shading represents the  $q$  assigned to each of three ancestral sources: Shoepack-strain (white), Leech-strain (grey), and Wisconsin-strain (black). Individuals were grouped based on  $q$  of Leech-strain heritage to show the strains increase over time.

In order to harvest muskellunge on Big Mantrap Lake they must be above the statewide 1219 mm TL limit that is currently in place. In the 2004 sample six fish or 13% of the total sample were above this with two of the fish having some shoepack-strain ancestry and four of them without (Table 2). The three pure shoepack-strain fish caught during the sample were not above the minimum harvestable size. Thirteen muskellunge or 10% of the 2013 sample were above the minimum length limit with the remaining 90%

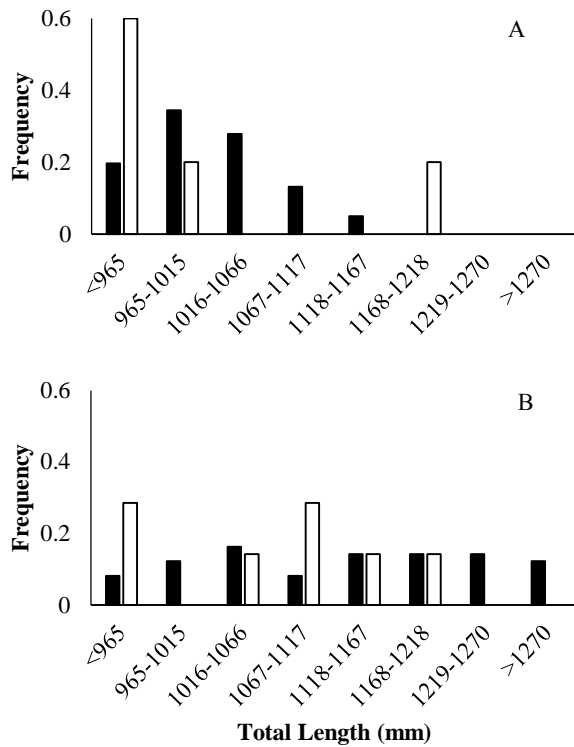


Figure 4- Frequency of length distributions (total length, TL) of male (A) and female (B) muskellunge from the 2013 Big Mantrap Lake, MN, modified Fyke net sample. White bars indicate fish with Shoepack-strain ancestry and black bars indicate fish without Shoepack-strain ancestry (i.e., Leech-strain, Wisconsin-strain, or Leech-strain x Wisconsin-strain individuals).

Table 2- Distribution of muskellunge above and below the 1219 mm TL minimum length limit during the 2004 and 2013 modified Fyke net sample on Big Mantrap Lake, MN. They are separated into fish with Shoepack-strain ancestry and fish without Shoepack-strain ancestry (i.e., Leech-strain, Wisconsin-strain, or Leech-strain x Wisconsin-strain individuals).

Strains	Under	Over
Shoepack ('04)	12 (26%)	2 (4%)
Other ('04)	29 (62%)	4 (9%)
Shoepack ('13)	12 (10%)	0 (0%)
Other ('13)	99 (80%)	13 (10%)

being below (Table 2). All of the individuals above the minimum length limit had ancestry from sources

other than Shoepack Lake, further demonstrating the effect of Shoepack-strain ancestry on fish size.

Mean total lengths (mm) were calculated for muskellunge samples by MNDNR biologists from 1993 to 2013. Since 1993, the mean TL (mm) for both male ( $P = 0.013$ ) and female ( $P = 0.003$ ) muskellunge has increased significantly (Figure 5). Mean total length for male muskellunge increased from 824 mm in 1993 to 1006 mm by 2013. Female muskellunge increased from a mean total length of 967 mm in 1993 to 1106 mm in the 2013 sample.

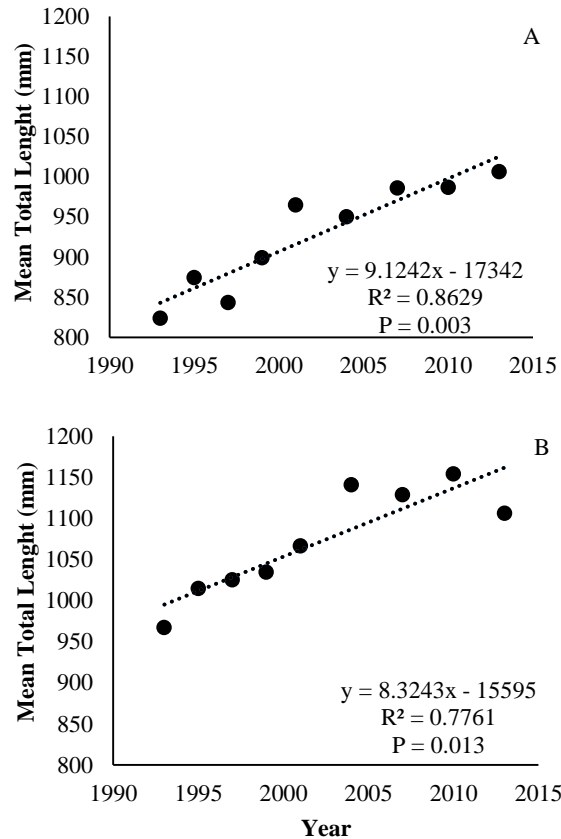


Figure 5- Mean Total Length (mm) of male (A) and female (B) muskellunge from modified Fyke net samples from 1993 to 2013.

## Discussion

In this study there was evidence to suggest a change in the genetics of the muskellunge population on Big Mantrap Lake. Our estimates showed a reduction in both mean and individual Shoepack-strain ancestry throughout sample years, inversely Leech-strain ancestry increased throughout the sample years. The overall decrease in Shoepack-strain ancestry over time can be contributed to a number of factors. Younk and Strand (1992) found a higher natural mortality rate for Shoepack Lake muskellunge when compared to the Leech-Strain.

This increased natural mortality rate coupled with continued stocking of Leech-strain fish into Big Mantrap Lake over time has led to the observed decline in Shoepack-strain fish.

Based on the observed Leech Lake source heritage among admixed individuals in the 2013 sample successful reproduction of Leech-strain individuals has occurred. Natural reproduction of stocked muskellunge has been documented in many lakes in Minnesota (Miller et al. 2012). The reproduction of Leech-strain fish in Big Mantrap Lake is an important step towards establishing a natural reproducing population in the lake.

Though there were indications of the effect of Shoepack-strain ancestry on fish size, no statistical differences in size distribution could be found. This contradicts Younk and Strand (1992) who found a shorter maximum calculated length for Shoepack Lake muskellunge when compared to Leech Lake muskellunge. These contradictory findings are more than likely attributed to one of three possibilities. The first is that our sample sizes of individuals with Shoepack Lake ancestry (males  $n = 5$ , females  $n = 7$ ) were not large enough to observe any significant changes in the TL distributions in either male or female fish. Secondly, due to the fact that no reliable ages could be attained we are unable to make significant length comparisons between age groups. We may have be categorizing fish of different ages into the same group. Third, Younk and Strand (1992) compared fish with pure strain ancestry. This is much higher than the maximum Shoepack-strain ancestry (55%) of any fish observed in this study, therefore the effect may have been lessened. It possible that all three of these factors have contributed to our contradictory results. The general increase in mean total length (mm) for both males and females as well as the increase in the number of fish with other ancestry above the minimum harvestable length point to positive impacts on growth rate for the Big Mantrap Lake muskellunge population throughout recent years. Miller et al. (2009) stated the decrease in maximum obtainable size for Shoepack-strain fish is seen as detrimental to the fishery, due to the fact that muskellunge fisheries are most often managed for trophy sized fish. For this reason the reduction in Shoepack-Strain ancestry observed in the 2013 sample is beneficial from both a managerial and angler standpoint.

*Management Implications-* The implications of this study for muskellunge management are significant. Currently for managers to actively change the ancestry of a muskellunge population they have three main options. The first would be a genetic purge where individuals are genetically screened and undesirable fish are eliminated. This process can only

be achieved efficiently if an adequate amount of individuals with a large proportion of undesired ancestry can be identified and removed (Miller et al. 2009). Though this option could have been considered for Big Mantrap Lake years ago, suitable genetic markers have only recently become available. The low number of individuals with Shoepack Lake ancestry and the low amount of Shoepack-strain ancestry they possess would now render this method ineffective and unnecessary. The second is to mitigate negative effects of further stocking by selecting individuals from within the population for brood stock. This would reduce further genetic risks from mixing populations (Miller and Kapuscinski 2003). Though this may have been possible at an earlier point in time on Big Mantrap Lake the lack of a remnant native population makes this option impossible. The third strategy involves “diluting” the effects of undesirable strains via an increase in stocking. Miller et al. (2009) suggests a strategy where Leech-strain individuals are used to stock the lake due to their proximity and success in Minnesota. This strategy was used on Big Mantrap Lake and appears to have been successful.

The results of this “dilution” may help to guide future management decisions when it comes to decisions of how to eliminate undesirable strains. The important management lesson to be taken away from Big Mantrap Lake is to be careful when stocking, especially when making decisions regarding source populations for the locations to be stocked. It is easier to prevent a problem than fix it when stocking (Miller et al. 2009). Jacobs et al. (1999) suggests that source strain evaluations conducted in common environments are the best way to detect genetic differences among populations. That is not to say that all effects of stocking will be easily quantified (i.e., the persistence of the Wisconsin-strain after one stocking event). The effects of stocking on traits that are more difficult to quantify such as disease resistance should always be considered (Miller et al. 2009). The success and impact of stocking may not always be as easy to quantify and may be hard to change once it has occurred. When stocking is being considered it is always best to proceed with caution.

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## Appendix A

Table A.1- All known muskellunge (MUE) stocking events on Big Mantrap Lake, MN. Included is year in which the stocking took place as well as the size (fry, fingerling (Fgl), yearling (Yrl)), number, pounds, and strain of these individuals are indicated in their respective columns.

<b>Date</b>	<b>Species</b>	<b>Size</b>	<b>Number</b>	<b>Pounds</b>	<b>Strain</b>
1920-42	MUE	Fry	54,000	N/A*	N/A*
1920-42	MUE	Fgl	32,211	N/A*	N/A*
1920-42	MUE	Yrl	5	N/A*	N/A*
1920-42	TME	Fry	15,700	N/A*	N/A*
1958	MUE	Fgl	1,246	N/A*	SHOEPAC
1959	MUE	N/A*	220	N/A*	SHOEPAC
1960	MUE	N/A*	3,934	N/A*	SHOEPAC
1961	MUE	Fry	25,900	N/A*	SHOEPAC
1961	MUE	Fgl	9,080	N/A*	SHOEPAC
1962	MUE	Fry	50,000	N/A*	SHOEPAC
1962	MUE	Fgl	4,650	N/A*	SHOEPAC
1963	MUE	Fgl	384	N/A*	SHOEPAC
1964	MUE	N/A*	8,297	N/A*	SHOEPAC
1966	MUE	N/A*	2,220	N/A*	SHOEPAC
1967	MUE	N/A*	80	N/A*	SHOEPAC
1968	MUE	Yrl	753	N/A*	SHOEPAC
1969	MUE	Fry	61,653	N/A*	SHOEPAC
1969	MUE	Fgl	1,735	N/A*	SHOEPAC
1970	MUE	Yrl	750	N/A*	SHOEPAC
1971	MUE	Fgl	2,065	N/A*	SHOEPAC
1972	MUE	N/A*	1,500	N/A*	SHOEPAC
1973	MUE	N/A*	1,675	N/A*	SHOEPAC
1974	MUE	N/A*	800	N/A*	SHOEPAC
1975	MUE	N/A*	992	N/A*	SHOEPAC
1976	MUE	Fry	20,000	N/A*	SHOEPAC
1976	MUE	Fgl	1,010	N/A*	SHOEPAC
1977	MUE	Fry	100,000	N/A*	SHOEPAC

Table A.1- continued.

<b>Date</b>	<b>Species</b>	<b>Size</b>	<b>Number</b>	<b>Pounds</b>	<b>Strain</b>
1978	MUE	Fry	94,000	N/A*	SHOEPAC
1978	MUE	Fgl	1,369	224	SHOEPAC
1979	MUE	Fgl	1,732	181	SHOEPAC
1980	MUE	Fgl	1,017	201	SHOEPAC
1981	MUE	Fgl	1,323	245.7	SHOEPAC
1982	MUE	Fgl	1,110	195	SHOEPAC
1983	MUE	Fgl	431	69	SHOEPAC
1987	MUE	Fgl	1,123	248.4	WISCONSIN
1988	MUE	Fgl	750	150	LEECH LAKE
1989	MUE	Fgl	750	150	LEECH LAKE
1990	MUE	Fgl	750	127.9	LEECH LAKE
1992	MUE	Fgl	750	214.3	LEECH LAKE
1994	MUE	Fry	18,688	N/A*	LEECH LAKE
1994	MUE	Fgl	750	290	LEECH LAKE
1996	MUE	Fgl	797	245	LEECH LAKE
1998	MUE	Fgl	831	453.6	LEECH LAKE
2000	MUE	Fgl	701	190	LEECH LAKE
2002	MUE	Fgl	722	267.4	LEECH LAKE
2004	MUE	Fgl	779	154.3	LEECH LAKE
2006	MUE	Fgl	750	271.3	LEECH LAKE
2008	MUE	Fgl	760	230	LEECH LAKE
2010	MUE	Fgl	750	230	LEECH LAKE
2012	MUE	Fgl	750	179	LEECH LAKE

\*N/A indicates that no information can be found.